Multi Residual Screening for the Determination of Organoflourine Pesticides in Farm Products Using High Performance Liquid Chromatography (HPLC); Tadem Mass Spectrometry (TMS)

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Abstract

Pesticide residues in aquatic products are of great concern due to the risk of environmental transmission and their extensive use in aquaculture. In this work, a quick screening approach was developed for the qualitative and semi-quantitative screening of 87 pesticide residues in aquatic products. The sample preparation was investigated, including extract solvent, extract methods, buffer salts, lipid removal, cleanup materials and filter membranes for aquatic products. Samples were extracted using a modified QuEChERS procedure, and two clean-up procedures were developed for UHPLC-Q/Orbitrap MS analysis based on the fat content of the aquatic products. The screening detection limits for all studied pesticides were distributed between 1 and 500 µg/kg in the three representative matrices. Seventy-one pesticides could be analyzed with a screening limit between 1 and 25 µg/kg in crayfish, sixty-one pesticides could be screened for limits between 1 and 50 µg/kg in crab. The accuracy results showed that recoveries ranged from 50 to 120% for 56 and 52 pesticides at medium-level for crayfish and crab, respectively. At high spiking levels, 65 and 59 pesticides were recovered within the range of 50–120% for the three matrices, respectively. The relative standard deviations of most compounds in different matrices were less than 20%. With this method, the local farmed aquatic products were tested for pesticide residues. In these samples, ethoxyquinoline, prometryn and phoxim were frequently detected. The majority of these confirmed compounds did not exceed 2.00 µg/kg. Two crabs with ethoxyquinoline at 200 µg/kg were detected, indicating the potential dietary risk.

Keywords: *pesticide residues; multi-residue screening; aquatic product; high-resolution mass spectrometry; sample preparation; QuEChERS; fish; crab; crayfish*

Introduction

Organofluorine pesticide is a type of novel, selective pesticide following organochlorine, organophosphorus and carbamate pesticides. Among these pesticides, fluxapyroxad, fluopyram, flubendiamide, flufenpyr-ethyl, penthiopyrad and cyflufenamid are the most widely applied (Chen, *et al.,* 2014; Zhang *et al.,*2014; Chen et al., 2012; Nagahori, *et al.,* 2009; Diehl, Busse, & Canvas, 2009; Yanase *et al.,* 2007; Shinsuke, *et al.,* 2007). The increasing use of organofluorine pesticides in agriculture has raised concerns regarding their fate in the environment. Organofluorine pesticides do not degrade easily in the environment and tend to bioaccumulate in organisms due to their lipophilic quality, then increase in concentration along the aquatic food chain (Murphy, *et al.,* 2012), eventually affecting human health. In response to this concern, the United Nations, European Union, Japan, CAC and China have stated the legal limits of Maximum Residue Limits (MRL) or tolerance level in agriculture products ((EU)No 491/2014 ; (EU) No 36/2014 ; (EU) No 737/2014 ; PMRL 2014-25).

The pressing need to determine the pesticide residues in aquaculture products at trace levels has improved the development of sensitive screening methods (Antonia, Diego, Paula, & Lorenzo, 2012). OFPs are mainly determined by liquid chromatography (LC) and LC-MS/MS methods (Chen, *et al.,* 2013 ; Gulkowska, *et al.,* 2014 ; Li, *et al.,* 2014). And multi-residue analysis methods are highly attractive for the simultaneous determination of several pesticides in one run which can be performed by significantly reducing the cost and time of analysis. However, the applications of these analytical methods to the real matrices of vegetable origin or water samples do not require particular cleanup approaches for the elimination of interferences present in those real samples (Nardelli, *et al.,* 2010). On the contrary, due to the matrix-dependent issues associated with co-extractive interference in real fish tissue samples contain a high amount of fat with respect to other agriculture products, and in fact they are very complex matrices consisting of fats, protein and pigments in different percentages. Despite several methods have been developed in recent years for the analysis of OFPs in agricultural products, none have been developed for fish tissue analysis. In the analysis of these samples, as a result, rapid and effective cleanup procedures in terms of analyte recovery, elimination of interferences and time are required for complex matrices in order to allow a reliable screening of contaminated samples.

Several approaches have been attempted to eliminate lipids and co-extracted interference from fatty food extracts, including solid-phase extraction (SPE), gel permeation chromatography, solid-phase microextraction (Doux, 2011), matrix solid-phase dispersion (MSPD) and dispersive-solid phase extraction (d-SPE). Among these methods, QuEChERS is the most used to reduce sample handling and processing time for analysis of pesticides in the fruit and vegetable samples (Grimalt et al., 2011). However, QuEChERS is not applicable to fish for the co-extractive interference of fats, proteins, fatty acids and lipids in the matrix, and SPE cleanup procedure is used to provide a baseline resolution for the analyte with a high detection signal and improved peak shapes, as well as facilitate efficient removal of matrix constituents, and reduce the noise level and risk for carry-over effects and column deterioration (Cho *et al.,* 2013). The aim of this work is the development and optimization of a multi-residue method based on solid-phase extraction (SPE) procedure and GC–MS/MS for the determination of six types of

OFPs in Garden eggs and chilly pepe. Different SPE cartridges, such as C18, Florisil, Al-N, NH2 and HLB, will be tested in order to minimize interferences and to achieve high recovery values. The performance parameters for the method validation, such as accuracy, precision, linear range, limit of detection and quantifications, will be determined by LC–MS/MS analysis of the standard solutions and spiked real samples.

EXPERIMENTAL

Reagents and materials

Thiofanox sulfon, thiometon, aldicarb sulfone, p horateoxon sulfoxide and sodium pentachloroph enol, Formic acid (HPLC grade, >98%), Ammonium formate (HPLC grade), anhydrous magnesium sulfate (MgSO4) and sodium chloride (NaCl) of analytical grade, Solid phase extraction (SPE) cartridges C18 (200 mg/3 mL) and primary secondary amine (PSA, 200 mg/3 mL) was purchased from Shanghai Sinopharm Reagent Group Co., Ltd. (Shanghai, China). HLB cartridges (200 mg/3 mL) was used. The dispersive solid phase extraction material, ethylenediamine-N-propyl silane (d-PSA), ODS C18 (d-C18) and Polar Enhanced Polymer (PEP), the disposable needle filter, hydrophobic polytetrafluoroethylene (H-PTFE, 0.22 μm) and nylon (Nylon, 0.22 μm), and the extraction salt package for QuEChERS (6 g $MgSO_4$ + 1.5 g Sodium acetate ($C_2H_3NaO_2$)) were all supplied by Shanghai Anpel Experimental Technology Co., Ltd.

Sample source

The aquatic products, namely crayfish and crab used for the method development and validation in this study, will be obtained from Damaturu Sunday market.

Solution Preparation

The stock solutions were prepared by dissolving an appropriate number of solid standards in methanol to obtain a 100 μg/mL concentration. The solutions were ultrasonicated or added with 0.1 mL of formic acid for the poorly soluble chemicals. These standard solutions were stored at -42 ◦C in darkness.

Sample Preparation

Samples (2.00 ± 0.02 g) was weighed in a centrifuge tube with the addition of 10 mL acetonitrile. Additionally, samples can be mashed with a glass rod before vortex if they are not well dispersed in acetonitrile. The salts, 2 g $MgSO_4$ + 0.5 g NaCl, was added after vigorous vortex for 5 min. The samples will then be treated with ultrasonication for 10 min and vortex for another 5 min. The supernatant was collected through centrifugation at 4000× *g* for 10 min (16RXII high-speed refrigerated centrifuge HITACHI CF, Japan). The extraction was repeated using 10 mL of acetonitrile on the residue following the procedure above, and the supernatant was mixed for further cleanup based on the differences between these matrices.

The supernatant was added with 300 mg $MgSO_4$, 150 mg PSA and 150 mg C18, and was mixed vigorously for 1 min for the Cleanup of low-fat aquatic products (fish, crayfish, etc.). Then, the supernatant was collected into a pear-shaped flask through centrifugation at 10,000× *g*, 5 ◦C for 10 min. This solution was evaporated to an approximate volume between 1 and 2 mL at 40 ◦C under vacuum. The concentrated solution was transferred to a 5 mL graduated glass tube, and the residue in the flask was washed with 3 mL acetonitrile and combined. After then, the concentrated solution was concentrated again to about 0.3 mL by mild nitrogen flow at 35 ◦C. The residue was dissolved and diluted with 1 mL of methanol-water (*v/v* 1:1), which was centrifuged at 3000× *g* for 10 min. After being filtered with

0.22 µm H-PTFE membrane, the sample was transferred into a vial for liquid chromatography analysis.

Instrument Method

Dionex Ultimate 3000 ultra-performance liquid chromatography-Q/Exactive orbitrap mass spectrometer (Thermo Fisher, MA, USA) was used for data acquisition under electron spray ionization at voltage 3200 V(+) or 2800 V(-) with sheath gas at 40 arb, auxiliary gas at 10 arb, sweeping gas at 1 arb and auxiliary gas heating temperature at 350 ◦C. The ion transport tube temperature was set at 325 ◦C. The mass spectrometry scan mode, Full Scan/dd-MS2(TopN) at scan range between 100 and 1000 *m/z*, mass resolution of 70,000 (Full MS) and 17,500 (MS/MS) was used at trigger threshold at 5 × 105 (Full MS) and 1 × 105 (MS/MS). The maximum injection time of 100 ms (Full MS) and 80 ms (MS/MS) with isolation window of 2.0 *m/z* was applied, where the top 2 strongest primary ions (TopN) were selected for secondary mass spectrometry acquisition.

Accucore^{TM} aQ -MS column (100 mm × 2.1 mm, 2.6 µm) was used for chromatography analysis at a flow rate of 0.3 mL/min and a temperature of 30 ◦C with an injection volume of 10 µL. The mobile phase A (0.1% formic acid and 5 mmol/L ammonium formate in water) and mobile phase B (0.1% formic acid and 5 mmol/L ammonium formate in methanol) were used with gradient elution procedure as follows: 0 min, 2% B, 0–4 min, 2–20% B, 4.0–5.5 min, 20–40% B, 5.5–10.5 min, 40–98% B, 10.5–12.9 min, 98% B, 12.9–15.0 min, 98–2% B, 15.0–20.0 min, 2% B.

Method Optimization

Optimization of Solvents, Salts and Additives

We investigated the extraction method to recover the multiple target compounds efficiently. The most common extraction solvents, acetonitrile and ethyl acetate, were chosen for optimization. The standard of pesticides was spiked in blank samples at 25 µg/kg or 50 µg/kg before extraction. Acetonitrile (10 mL) once, acetonitrile (10 mL) twice and a combination of acetonitrile (10 mL, once) and ethyl acetate (10 mL, once) for the extraction were compared in the selection of solvent. Then, the recoveries of the target compounds were compared with or without the addition of 4 g MgSO4 + 1 g NaCl and 6 g MgSO4 + 1.5 g C2H3NaO2. Furthermore, the amounts of salt (MgSO4 + NaCl), 4 g MgSO4 + 1 g NaCl, 2 g MgSO4 + 0.5 g NaCl and 0.5 g MgSO4 + 0.5 g NaCl, were examined for their effect in recoveries, respectively. The concentration of the acidified extractant was determined by adding several concentrations of formic acid (0%, 0.1%, 0.5% and 1%) under optimized parameters of solvent and salt in the section of additives optimization.

Optimization of Cleanup

In this study, the aquatic products were divided into two categories according to their fat content. A total of 5% fat content was used to classify high-fat and low-fat fishery products. Different cleanup methods were examined according to their differences in matrices. Fat-rich aquatic products, such as crab, large yellow croaker and eel, were investigated as representative fat-rich matrices. Other fish and shrimp were classified as low-fat aquatic products. In terms of low-fat fishery products, five dispersive solid phase extraction materials (d-PSA+d-C18, d-PSA, PEP, d-PSA+PEP, chitosan) were investigated by evaluating the cleanup effects at the spiked concentration of 25 µg/kg.

The uptake of pesticides by hexane was evaluated by direct co-extraction of a mixed standard solution with 2 mL of hexane. Next, in order to minimize the uptake of the targets by hexane during the degreasing process, the lipid removal with 2 mL, 5 mL and 10 mL of hexane in the extract or 3 mL of hexane in the concentrated extract by rotary evaporation was studied. Four

solid-phase extract materials (C18, d-PSA+d-C18, HLB and PSA) were examined with crab matrix to obtain better efficiency after the cleanup step with hexane. Then, different volume ratios of acetonitrile-water (60/40, 70/30, 80/20, 90/10, 100/0) were examined on the recoveries of the intensities of these compounds, because a tiny amount of fat cannot be well dissolved with methanol–water solution (1:1, *v/v*).

Optimization of Filter Membrane

Filter membrane is widely used to ensure particulate-free status in the reconstitution solution. We evaluated different filter membranes for their adsorption profile of target compounds. Five syringe filters with the pore size of 0.22 µm (hydrophilic poly tetrafluoro-ethylene (H-PTFE), nylon, poly tetrafluoro-ethylene (PTFE), poly vinylidene fluoride (PVDF), poly ether-sulfone (PES)) were tested on the crayfish blank extract spiked with target compounds at 100 µg/kg.

Method Validation

Matrix Effect (ME)

Different aquatic products was treated according to the procedure described earlier and the matrix extract was obtained with samples free of the above targets. The evaluation of ME was performed as previous studies with consideration of multiple targets (Huang, *et al., 2019:*) Matrix-matched standard solution and solvent standard solution at 100 ng/mL were prepared by diluting the concentrated mixed standard solution of 500 ng/mL with the blank matrix solution and the methanol–water solution (1:1, *v/v*), respectively. These two solutions were analyzed with HPLC-HRMS and the peak areas were measured as Ab and As, respectively. The matrix effects (ME) of different sample preparation methods for aquatic products were analyzed according to the formula ME(%) = (1 - Ab/As) × 100%.

Screening Detection Limit (SDL)

Different aquatic products (2.00 ± 0.02 g) were weighed and added with mixed standards solutions to obtain spiked concentrations at 1, 5, 25, 50, 100 and 200 µg/kg, respectively. The spiked samples were prepared with six replicates for each spiking level, which were thoroughly mixed and silent for 20 min. These spiking samples were extracted, cleaned and analyzed to examine the detected compounds at different spiking levels. The SDLs were determined in compliance with the requirements of SANTE/11312/2021, which involve analysis of at least 20 samples spiked at the estimated SDL, with slight modifications. As demonstrated in previous studies, the SDLs were set at the lowest spiking concentration, where the targets could be detected in all six replicates (portoles, *et al.*, 2017: De paepe, *et al.*, 2019).

Accuracy and Precision

The concentrations for method validation of different matrices were prepared according to their SDLs for different compounds. The spiked samples at 1, 5, and 50 µg/kg for crayfish and 5, 25 and 100 µg/kg for crab were prepared, respectively. Six replicates for each concentration level in different matrices were prepared in accordance. The blank extracts of different matrices, matrix-matched standard solutions and solvent-matched mixed standard solutions were prepared and analyzed simultaneously with these spiked samples.

RESULTS AND DISCUSSION

Optimization of Extraction Methods

Aquatic products are more complex than water and substrates due to the different biochemical characteristics of the matrices. Interactions between target compounds and substrates may result in different extraction efficiencies for multiple targets. The results of the extraction solvent optimization showed that the three extraction methods detected similar amounts of compounds. The three extraction methods (acetonitrile once, acetonitrile twice and

acetonitrile × ethyl acetate) identified 74 and 73 pesticides, respectively. However, the recoveries were much higher when the extraction was performed twice with acetonitrile than when it was performed once. Twenty-four compounds showed an improvement of more than 10% in recoveries. Meanwhile, 5 pesticides (acephate, doramectin, aldicarb, thiophanate-ethyl and malathion), which were extracted twice, showed a decrease of less than 10% in recoveries compared with those that were extracted once. This may have resulted from an increase in concentration time due to a larger volume of extract with an extra extraction. The effect of combining acetonitrile and ethyl acetate was comparable to that of employing acetonitrile twice. Considering that ethyl acetate has superior lipid solubility and will remove more non-polar interferents, such as fat, leading to a more significant matrix impact and more difficult cleanup Operation acetonitrile extraction was chosen for a two-step approach.

Salts

Some pesticides, such as acephate with a log K_{ow} of -0.85, are very hydrophilic. The amount of water in the matrix may result in less effective extraction. Regarding the first optimization of salt, 70, 74 and 73 compounds were confirmed for without salt, 4 g $MgSO_4$ + 1 g NaCl and 6 g $MgSO_4$ + 1.5 g $C_2H_3NaO_2$ at the spiking concentration of 25 µg/kg, respectively. There was no obvious improvement in terms of the detected number. However, in terms of the recoveries, the addition of $MgSO_4$ + NaCl lead to slight or moderate loss of recoveries for most of the pesticides. Conversely, doramectin, ivermectin B1a, aldicarb and malathion were not detected at all without salt. Thus, the addition of salt ($MgSO_4$ + NaCl) was necessary and salt usage needs further investigation. It can be observed from Figure 1 that too much salt may result in loss of the target and cause low recovery ratio. The results of salt usage showed that 68% of the compounds were extracted with more than 10% improved recoveries for the use of 2 g $MgSO_4$ + 0.5 g NaCl in the further investigation. The use of 0.5 g $MgSO_4$ + 0.5 g NaCl could not efficiently remove water, resulting in a loss in the extraction process. Therefore, the best salt combination, 2 g $MgSO_4$ + 0.5 g NaCl, was finally used for water removal

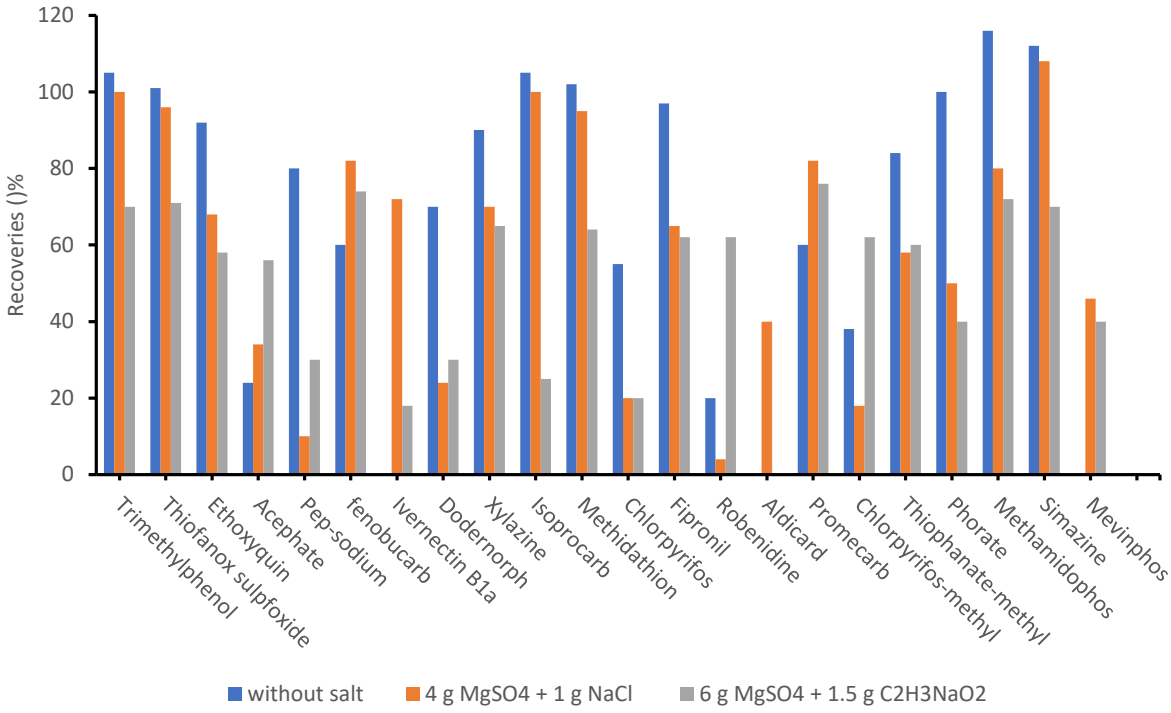

Figure 1. Typical compounds with significant differences in extraction efficiency of target compounds under different salts combination.

For some pesticides, a certain amount of acid in the extractant may be beneficial to the stability of the pesticide (Sandín-España *et al., 2022*). In our work, Dodemorph, xylazine, robenidine, phorate and avermectin B1a may be extracted with greater than 20% recoveries using 0.5% or 1% formic acid acetonitrile, according to the experiment's results (Figure 2). However, the presence of formic acid might cause the breakdown of ethoxyquin, resulting in less than 10% recovery. Furthermore, the matrix effect of aminocarb was too high in the acid-containing extraction solution, which masked the target and made it undetectable. Moreover, nearly all of dioxacarb was lost in 1% formic acid-acetonitrile. Therefore, the use of formic acid to improve the recoveries was unreasonable, and acetonitrile was set as the extraction solvent.

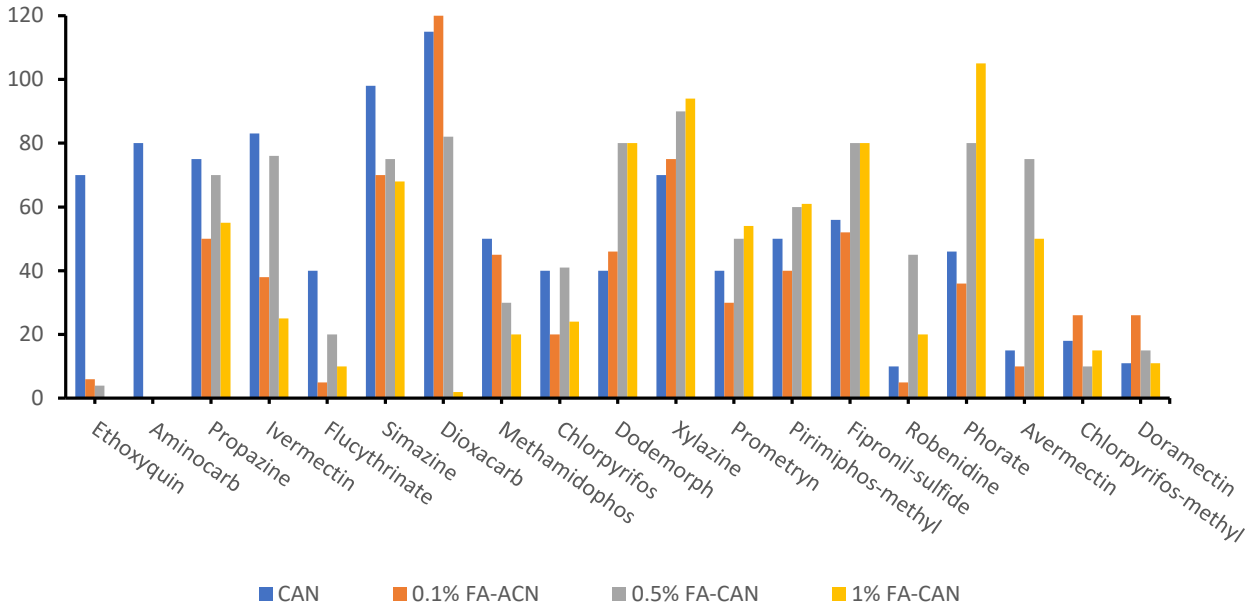

Figure 2: Influence of the formic acid (FA) content on representative compounds.

Optimization of the Cleanup Procedure

Low-Fat Fishery Products

The target recoveries **(Figure 3a**) and ME (Figure 3b) of crab were investigated. The number of detected compounds under the individual treatment of the five materials (dPSA+d-C18, d-PSA, PEP, d-PSA+PEP, chitosan) was 76, 74, 73, 73 and 72, respectively. Compared to the ME results, more than 60% of the compounds showed ME in the range of -40% to 40% after the treatment of d-PSA+d-C18. The other treatment showed less than 40% of the compounds within this ME range. In terms of recovery, most compounds with recoveries below 50% were found in the PEP cleanup, while most with recoveries in the range of 70–120% were identified with the use of d-PSA. The use of d-PSA+d-C18 demonstrated more than 80% of compounds with recovery between 50 ad 120%. According to the above results, the combination of d-PSA and d-C18 was more suitable for the clean-up of low-fat aquatic matrices for screening the selected pesticides.

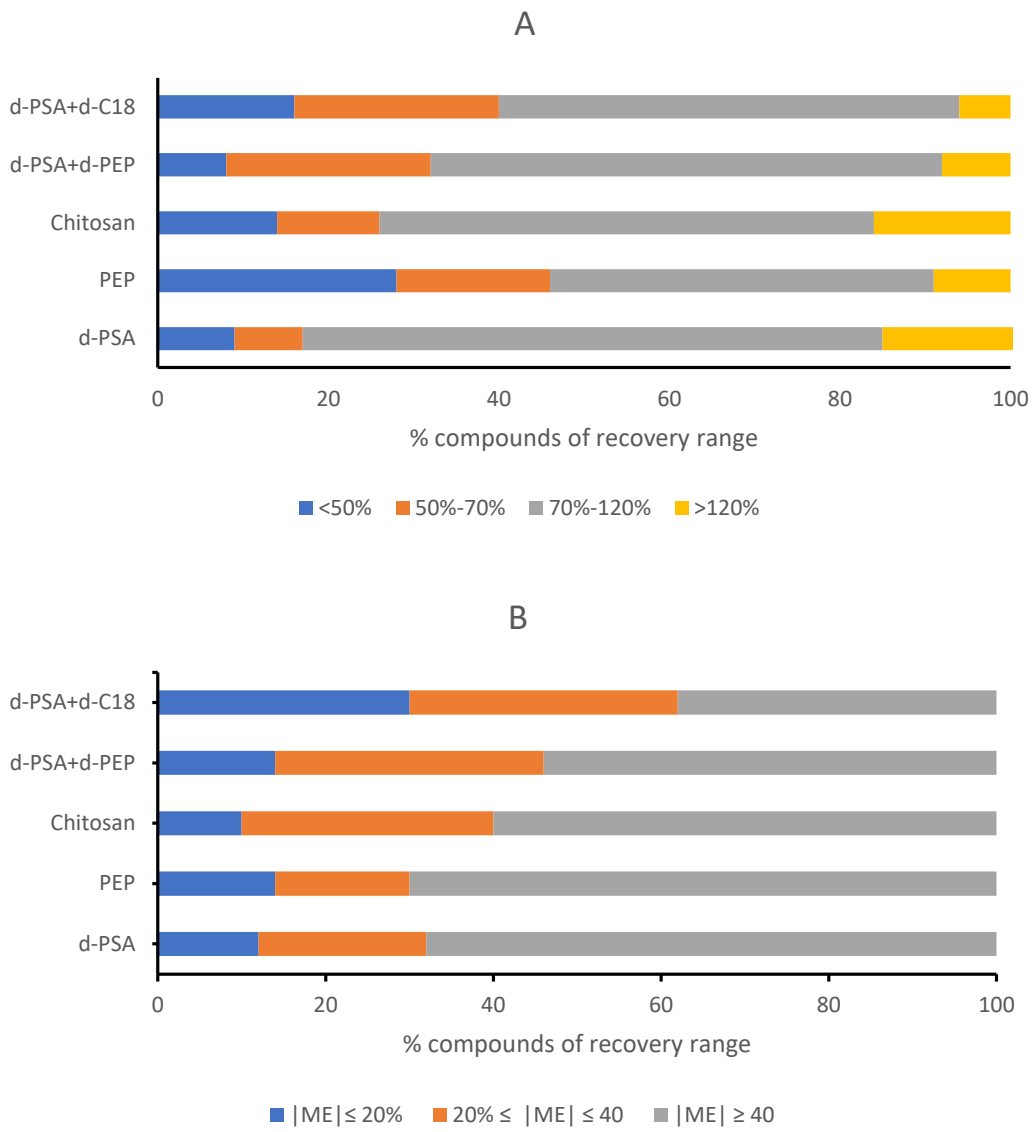

Figure 3: Proportion profile of compounds of different ranges of recoveries (a) and matrix effect (b) under five different cleanup treatments for low fat aquatic products

Fat-Rich Aquatic Products

It is well known that the fat extracted in solvent would greatly affect the recoveries of target analyte and result in a noticeable matrix effect during analysis. The co-extract of hexane on the target compounds during fat removal was exami ned for the fat rich aquatic product.

Eleven analytes, including dodemorph, doramect in, cyfluthrin, flumethrin, taufluvalinate, fenvalerate, deltamethrin, thiophanate-methyl, thiophanate-ethyl, bifenthrin and tributyl phosphorotrithioate, were shown to lose 53.5% to 93.0% of their peak intensity in the co-extract. Therefore, lipid removal by hexane needs to be optimized. The numbers of detected pesticides by the four different ways (2 mL, 5 mL, 10 mL and 3 mL of hexane) of lipid removal were 62, 64, 63 and 67, respectively. The lipid removal after

concentrating by evaporation showed more detected compounds. Furthermore, compared with lipid removal before evaporation, more than half of the chemicals showed a 21% increase in recoveries by this approach of lipid removal. Finally, the removal of lipids following evaporation reduced the loss of pesticides; thus, post-evaporation lipid removal was chosen for further clean-up.

The detectable compounds after the individual use of C18, d-PSA+d-C18, HLB and PSA were 63, 63, 60 and 64, respectively. HLB allowed fewer compounds to be detected and, thus, resulted in poor recoveries of compounds after cleanup. PSA and C18 showed similar recovery profiles using d-PSA+d-C18 (Figure 4). In contrast, PSA showed lower recovery losses for propetamphos, aldicarb sulfoxide, macbal and xylazine. This result indicates that PSA is preferred for the subsequent cleanup procedure. However, more than half of the target compounds had better lipid solubility. They could co-exist with the residual fat. If these analytes are not well dissolved, they could be lost in the following analysis on the HPLC-HRMS. Acetonitrile has better lipid solubility than methanol, and can also avoid the loss of weakly polar compounds in redissolution with methanol. With the increase in acetonitrile proportion, the fat residue gradually dissolved in the reconstitution solution, further leading to the improved recoveries of thirty-seven compounds, despite the fact that five compounds were found with decreased recoveries at 12~68% (Figure 5). Generally, the best recoveries profile was found at the ratios 80/20 and 90/10 of acetonitrile–water solution. The optimized reconstitution solution demonstrated 15 compounds with more than 30% improved recoveries. The recoveries of 48 and 47 compounds with the acetonitrile water solution (80/20 and 90/10) ranged between 50% and 120%, compared to 39, 43 and 44 compounds with the other acetonitrile–water solutions (60/40, 70/30 and 100/0). However, both ratios 80/20 and 90/10 of acetonitrile-water showed compounds with significantly increased or decreased recoveries as the organic phase increased. For example, in the acetonitrile–water solution (80/20), propamocarb, methamidophos and aldicarb sulfoxide had more than 18~35% higher recoveries than in the acetonitrile–water solution (90/10), while propetamphos, robenidine and carbaryl had more than 20~30% decreased recoveries in the same solution. Finally, the acetonitrile–water solution (85/15) was used as the reconstitution solution for the fat-rich aquatic product in order to provide a balanced, acceptable recovery profile.

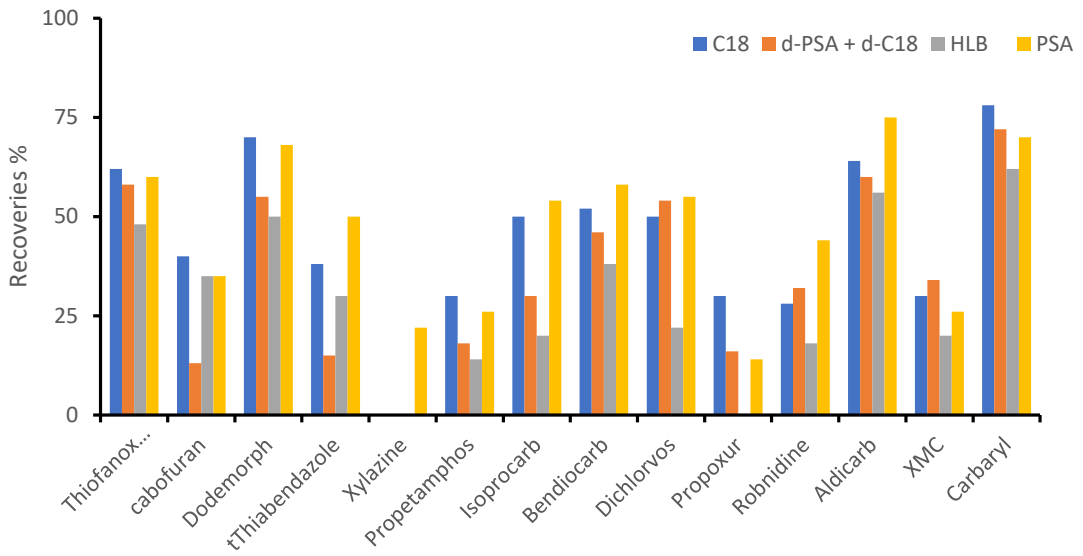

Figure 4. the cleanup efficiency with significant differences of target compounds in fat-rich aquatic product

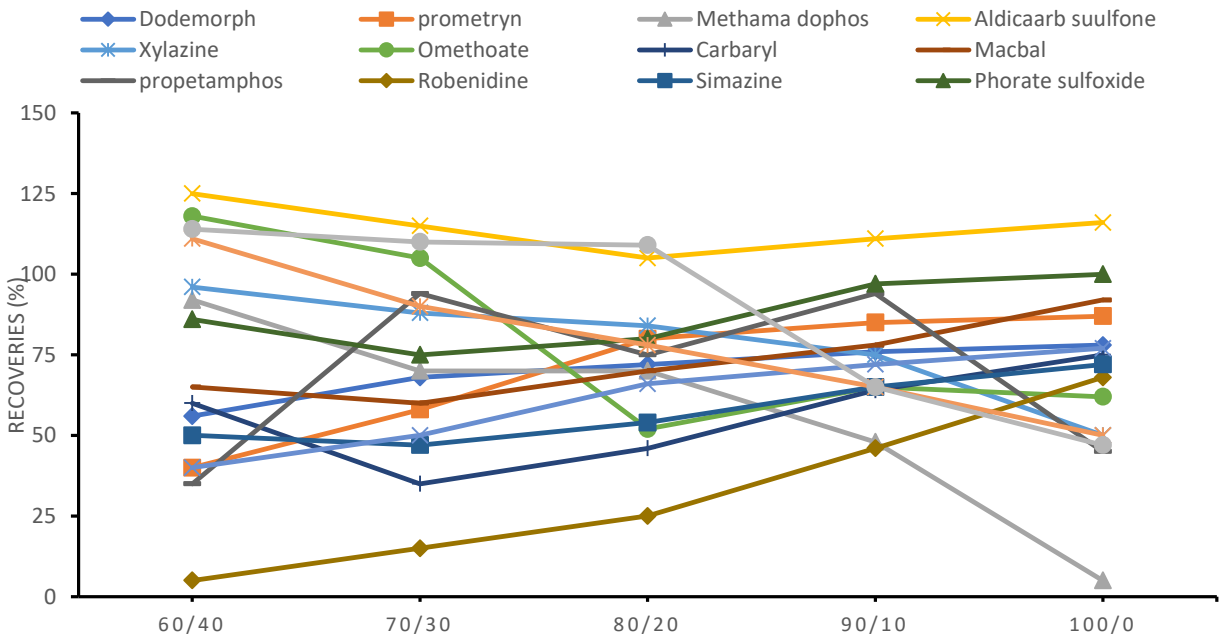

Figure 5. Effect of the ratio between acetonitrile and water (acetonitrile/water, v/v) as reconstitution solution on recoveries profile of 15 representative compounds.

Effect of Filter Membrane

The analytes could be adsorbed on the specific membrane if it was not well examined. Therefore, the adsorption profile of target compounds of five syringe filters was compared in our work. Using PES membrane, significant analyte loss occurred

for methamidophos, aldicarb, robenidine and deltamethrin. PES is usually suitable for filtering in hydrophilic solvent. It may also adsorb hydrophilic compounds, such as robenidine and deltamethrin. A slow filtering rate may also result in adsorption of less polar analytes. In general, it is agreed that PES is unsuitable for the filtration of all these targets of interest. The PDVF membrane, meanwhile, exhibited stronger adsorption for the less polar pesticides, such as flucythrinate and deltamethrin. Although nylon is suitable for filtering in both aqueous and organic solvents, simazine, simetryne, carbaryl, aldicarb, propoxur and sodium pentachlorophenolate were lost significantly on nylon with less than 50% recoveries. It was noticed that H-PTFE adsorbed less compounds and could ensure the recoveries of sixty-six compounds in the range of 90–110%. Less than 60% of these compounds were observed with recoveries in the range of 90–110% for other filter membranes (Figure 6). The results also showed that H-PTFE with the hydrophilic treatment had fewer adsorption losses than PTFE for a wider polar range of multi-targets. Therefore, a H-PTFE syringe filter was chosen to treat the reconstituted sample solution before analysis.

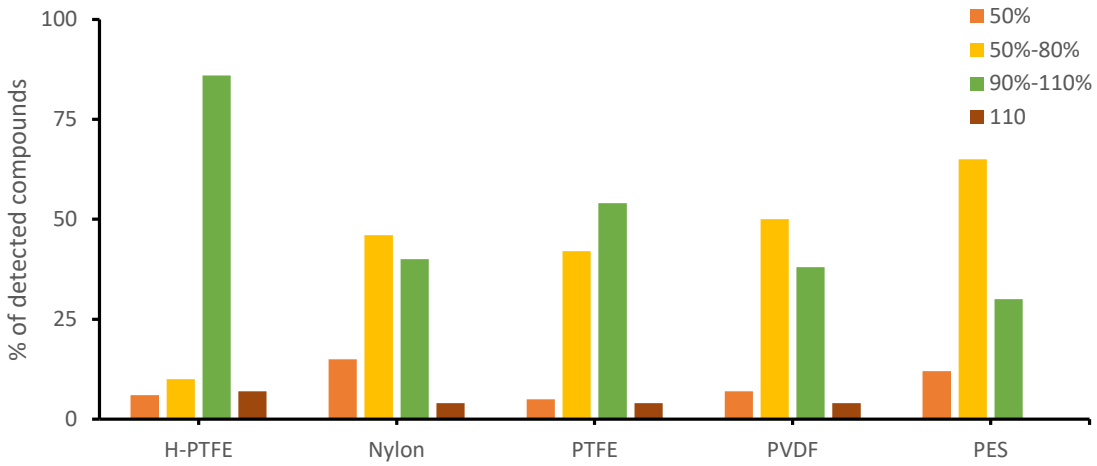

Figure 6. The percentage distribution of the compounds with different recovery profiles after filtering the reconstituted solution with different membranes.

Method Performance

Matrix Effect

The matrix effect of this method was evaluated on two representative aquatic products, typically in crayfish and crab. After treatment with the optimized procedure on the blank samples, a concentration of 100 ng/mL was prepared by diluting the concentrated solution with the blank solution. The acquired signal of the matrix-matched standards was compared with the solvent standards. Results showed that more than 80% of the compounds displayed the matrix suppression effect in the range of -40% to 40% in blank crayfish extract. Four compounds were found with a suppression effect of over 40%, including acetamiprid, methamidophos, aldicarb and flumethrin. A matrix enhancement effect of more than 40% was observed for imidacloprid, which may be due to the residual pigments in the solution (Ncube, 2019). As for the fat-rich samples, i.e., crab, the matrix suppression effect was usually observed. More than 20% of the

target compounds showed suppression effects between -40 and 40%, and around 40% of the compounds had more than 60% matrix suppression. It may have been attributed to the high-fat content, which still existed even after the cleanup steps, competing with the analytes for ionization, and finally leading to a suppression effect. However, as a screening method, the priority was to detect as many targets as possible simultaneously. Although apparent matrix effects could still be observed, the current result was sufficient for a semi-quantitative analysis.

Screening Detection Limits

To evaluate the performance of the developed method, SDLs for these compounds in different matrices are crucial parameters for practical use. Eighty and eighty-one compounds were found with SDLs in the range of 1–100 µg/kg crab and crayfish, respectively (Figure 7). Seventy-three compounds could be screened out for the crab matrix, with their limits ranging between 1 and 500 µg/kg. At the spiking concentration of 1 µg/kg, 52, 44 and 34 compounds were screened out and confirmed in crayfish and crab, respectively, while 63, 62 and 46 target compounds could be confirmed at 5 µg/kg. At 25 µg/kg, 70 and 62 compounds were confirmed in crayfish and crab, while at 100 µg/kg, the number of detected compounds in the three matrices reached 80, 81 and 67, respectively. For the crab matrix, 73 compounds were identifiable at 500 µg/kg.

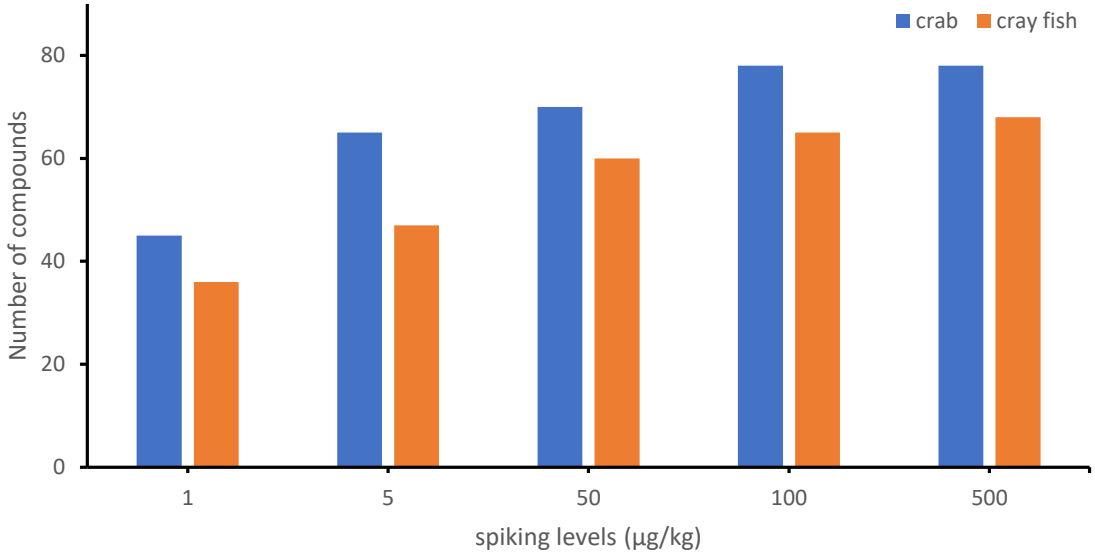

Figure 7 the number of detected compounds in crayfish and crab at different spiking concentrations.

Conclusions

This work established a quick screening technique for 87 pesticides in aquatic products based on high-performance liquid chromatography-tandem quadrupole-orbitrap mass spectrometry. The extraction and cleanup procedures were optimized for different aquatic products, making the method more sensitive and accurate, with more target compounds to be analyzed in a high-throughput way. The evaluation of the proposed method was performed at the matrices of crayfish and crab,

and SDLs between 1 and 100 µg/kg, matrix effect between -40 and 40%, recoveries between 60 and 120%, and RSDs of less than 20% of the majority of these compounds were obtained in the three matrices, respectively. Furthermore, the method was employed in the investigation of pesticide residues in farmed aquatic products consumed in Damaturu. The screening results revealed that the compounds frequently detected in farmed aquatic products were mainly insecticides and herbicides used in agriculture. In addition, the noncompliant addition of ethoxyquin in the feeds may introduce the exceedance of EU standard limits for farmed fish. Therefore, the regulation of farmed fishery feeds in Nigeria must be strengthened. This study demonstrated the possible occurrence of pesticide residues and their metabolites in the local aquaculture environment and in aquatic products, posing potential hazards to the safety of aquatic products. Therefore, a new screening approach for pesticide residue monitoring was presented with excellent efficiency, stability and accuracy.

Acknowledgement

The authors wish to acknowledge the Tertiary Education Trust Fund (TETFund) for sponsoring this research.

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